HHS Public Access

Author manuscript

Cell Tissue Res. Author manuscript; available in PMC 2015 March 31.

Published in final edited form as:

Cell Tissue Res. 2008 October ; 334(1): 37–45. doi:10.1007/s00441-008-0664-y.

Cadherin-7 Function in Zebrafish Development

Qin Liu[1,*], **James A. Marrs**[2], **Richard L. Londraville**[1], and **Amy L. Wilson**[1]

[1]Department of Biology, University of Akron, Akron, OH 44325

[2]Department of Medicine, Indiana University Medical Center, Indianapolis, IN 46202

Abstract

Cadherin cell adhesion molecules play crucial roles in vertebrate development. Most studies have focused on examining functions of classical type I cadherins (e.g. cadherin-2) in the vertebrate development. There is little information on the function of classical type II cadherins (e.g. cadherin-7) in vertebrate development. We previously showed that *cadherin-7* mRNA exhibited a dynamic expression pattern in the central nervous system and notochord in embryonic zebrafish. To gain insight into cadherin-7 role in the formation of these structures, we analyzed their formation in zebrafish embryos injected with *cadherin-7* specific antisense morpholino oligonucleotides (MO). Notochord development was severely disrupted in MO-injected embryos, whereas gross defects in central nervous system development were not detected in MO-injected embryos. Our results show that cadherin-7 plays an important role in the normal development of the zebrafish notochord.

Keywords

cell adhesion molecules; notochord; central nervous system; antisense morpholino oligonucleotides

Introduction

The vertebrate notochord, derived from the chordamesoderm, is an important source of molecules (e.g. shh) crucial for development of various tissues and organs including the neural tube and somites (Smith, 1993; Bumcrot and McMahon, 1995). In zebrafish, soon after specification of the notochord precursor domain (5-6 hours post fertilization (hpf), Melby et al., 1996, 1997), morphogenetic movements including convergence and extension accompanied by mediolateral intercalation behavior in the dorsal mesodermal cells results in the formation of a straight, rod-shaped notochord structure along the dorsal embryonic midline at 12 hpf (Glickman et al., 2003). Molecular mechanisms underlying vertebrate notochord development have been under intense investigation. Some genes, such as *Xenopus Xnot* (*Cnot*, *Noto* and *floating head* as its chicken, mouse and zebrafish orthologs, respectively) and zebrafish *no tail* (von Dassow et al., 1993; Halpern et al., 1993; Stein and Kessel, 1995; Talbot et al., 1995; Odenthal et al., 1996; Beckers et al., 2007), are shown to

[*]To whom correspondence should be addressed: Phone: 330-972-7558; Fax: 330-972-8445; qliu@uakron.edu. (qliu@uakron.edu) (jmarrs@iupui.edu) (londraville@uakron.edu) (amy45@iwon.com)

play crucial roles in early differentiation of the notochord, while other genes, such as *grumpy* and *sleepy* (Odenthal et al., 1996; Parsons et al., 2002), are involved in late notochord development. Cadherin cell adhesion molecules have also been implicated in the formation of the notochord (see below).

Cadherins are a family of transmembrane proteins that mediate cell-cell adhesion mainly through homophilic interactions (Takeichi, 1991). Classical type I cadherins (e.g. cadherin-1, cadherin-2 and cadherin-4, Nollet et al., 2000) play crucial roles in the formation of vertebrate tissues and organs including the notochord and central nervous system (CNS) (Redies and Takeichi, 1996; Gumbiner, 2005). There is little information on the function of other cadherins (e.g. cadherin-6 and cadherin-7, members of the type II cadherins, Nollet et al., 2000) in the development of the vertebrate notochord and CNS. In this study we showed that cadherin-7 message (*cdh7*) and protein (Cdh7) were expressed by the notochord in embryonic zebrafish, and interfering with cadherin-7 function using translation blocking morpholino antisense oligonucleotides severely disrupted the notochord development, suggesting that normal formation of the notochord requires cadherin-7 function. In contrast, gross defects in CNS development were not evident in *cdh7* morphant embryos comparing various CNS differentiation markers.

Materials and Methods

Zebrafish

Zebrafish (*Danio rerio*) were maintained as described in the Zebrafish Book (Westerfield, 2000). Zebrafish embryos were obtained from breeding of wild-type adult zebrafish. Embryos for whole-mount immunocytochemistry or in situ hybridization were raised in PTU (1-phenyl-2-thiourea, 0.003%) to prevent melanization. All animal-related procedures were approved by the Care and Use of Animals in Research Committee at the University of Akron.

MO injections and mRNA synthesis

Two translation blocking morpholino antisense oligonucleotides (cdh7MO1 (ATG MO): 5′-GCC AAC AGC AGT ATA GTC ATT ACA G-3′, cdh7MO2 (UTR MO): 5′-CAG AGC CAA TCT GCA TCA TTT GTT G-3′, and a MO with five-mismatched nucleotides (5-mis cdh7MO1: 5′-GCg AAg AGg AGT ATA GTg ATT AgA G-3′) were used in the study. These MOs, designed by and purchased from Gene Tools (Philomath, OR), were used as described (Nasevicius and Ekker, 2000). Compared with data bases using BLAST, the *cdh7* MOs sequences showed no significant similarities to any sequences other than zebrafish *cdh7* (GenBank accession number: DQ411036). MOs were microinjected into one- to four-cell stage embryos at 2 nl (Table 1) in Daneau buffer (58 mM NaCl, 0.7 mM KCl, 0.4 mM $MgSO_4$, 0.6 mM $Ca(NO_3)_2$, 5.0 mM HEPES pH 7.6).

The zebrafish *cdh7* (12 nucleotides in the 5′UTR + coding region) was amplified with primers containing NcoI restriction sites and cloned into pCS2+MT (myc tag, Turner and Weintraub, 1994). The PCR product was verified by restriction digestion and sequencing. Capped *cdh7* mRNA was synthesized from the pCS2+MT/cdh7 vector using SP6 mMessage

mMachine kits (Ambion, Austin, TX). *cdh7* mRNA (75 to 150 pg/embryo) was injected alone or with the cdh7MO2 (*cdh7* mRNA sequence does not contain binding sites for cdh7MO2) into one- to four-cell stage embryos as described above.

Injected embryos were allowed to develop at 28.5°C until the embryos reached desired stages (e.g. 50 hpf), anesthetized in 0.02% MS-222 and fixed in 4% paraformaldehyde and processed for in situ hybridization (ISH) or immunocytochemistry (ICC, see below).

Cadherin-7 antibody production

A synthetic peptide 5′-DRNTDLERFFNIESPTG-3′, corresponding to zebrafish cadherin-7 amino acid residues 370-386 (Liu et al., 2007), was conjugated to keyhole limpet hemocyanin and used to immunize two rabbits (Covance Research Products, Inc., Denver, PA). The resulting crude rabbit polyclonal antiserum was affinity purified by covalently linking the synthetic peptide to Affi-Gel 15 resin (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. Detailed procedures for the affinity purification were described previously (Liu et al., 2001a,b).

In situ hybridization, immunoblotting and immunohistochemistry

Detailed procedures for ISH, ICC and immunoblotting were described previously (Liu et al., 1999; 2001a). ISH probes used were *cdh7* (Liu et al., 2007), *dlx2a* (Akimenko et al., 1994), *krox20* (Oxtoby and Jowett, 1993), *pax7* (Seo et al., 1998), *shh* (Krause et al., 1993). Primary antibodies used were anti-acetylated tubulin (1:2,000; Sigma, St. Louis, MO), anti-HuC/HuD (1:2000; Molecular Probes/Invitrogen, Carlsbad, CA), cadherin-7 (1:2,000 and 1:3,000 for immunoblotting and immunofluorescent methods, respectively), zn5 (1:1,500; Zebrafish International Resource Center, University of Oregon, Eugene, OR), and 3A10 (1:500; The Developmental Studies Hybridoma Bank, The University of Iowa, Iowa City, IA).

Results and Discussion

Cadherin-7 expression in the developing notochord

Cadherin-7 message *(cdh7)* expression was first detected in the developing zebrafish nervous tissue at 12 hpf (Liu et al., 2007). *cdh7* was not detected in the notochord of 15 hpf embryos (Fig. 1A), but its expression was found in the notochord of 17-18 hpf embryos (Fig. 1B). *cdh7* expression in the notochord continued in 20-21 hpf embryos (data not shown), but *cdh7* expression was not observed in older embryos (24 hpf, Fig. 1C, 36-50 hpf, data not shown). Cadherin-7 protein expression (Cdh7) was similar to its message: strongly expressed in the notochord of 18-21 hpf embryos (Fig. 1F), and it was much reduced in 24 hpf notochord (Fig. 1G), and was not detectable in notochord of older embryos (26-30 hpf, data not shown). Specificity of the cadherin-7 antibody was demonstrated by immunoblotting using zebrafish embryonic tissues (lysates of whole embryos at 30-36 hpf). The antibody detected an intense band at about 100 kDa (Fig. 1D, lane 1), a molecular mass similar to the mouse cadherin-7 protein (105 kDa, Nakagawa and Takeichi, 1998). The zebrafish cadherin-7 antibody also detected a faint band about 110 kDa, a molecular mass similar to several type I classical cadherins (Liu et al., 2001a,b). The staining of both bands

was eliminated when the antibody had been preincubated with the synthetic cadherin-7 peptide used for the generation of the cadheirn-7 antibody (Fig. 1D, lane 2). Moreover, the antibody staining pattern in the embryonic brain (Fig. 1E) was similar to *cdh7* expression in the brain (Liu et al., 2007, Figs. 5 and 6).

Despite the large number of cadherins isolated (Nollet et al., 2000), only a few cadherins (cadherin-2, cadherin-10, EP-cadherin, axial protocadherin, and protocadherin Fat) have been found to be expressed in the developing notochord (Levi et al., 1991; Simonneau et al., 1992; Nakagawa and Takeichi, 1997; Kuroda et al., 2002; Down et al., 2005; Liu et al., 2006). *cdh7* expression in the embryonic zebrafish notochord was brief (several hours), compared to its expression in the CNS (3 days). This brief *cdh7* expression in the embryonic notochord is similar to that of *axial protocadherin* in embryonic *Xenopus* (Kuroda et al., 2002), and *cadherin-10* in developing zebrafish (Liu et al., 2006).

Cadherin-7 is involved in the notochord development

Morpholino antisense oligonucleotide (MO) techniques effectively and selectively block gene function in zebrafish (Ekker, 2000; Nasevicius and Ekker, 2000). Injections of translation blocking *cdh7* MOs into one- to four-cell stage zebrafish embryos resulted in embryos (*cdh7* morphants) with undulating bodies (Figs. 2 and 3; Table 1). The specificity of *cdh7* MOs was demonstrated by rescuing the phenotype with synthetic *cdh7* mRNA injections (Fig. 2D; Table 1). Moreover, Cdh7 expression in the notochord was greatly reduced in *cdh7* morphants (Fig. 2G and H) compared to uninjected control embryos (Fig. 2E), or control MO (5-mis) injected embryos (Fig. 2F).

The body undulation, first detected in 18 hpf embryos (Fig. 3F), was both dorsoventral and lateral, and it became more obvious at 22-24 hpf (Fig. 3G). The phenotype persisted in older embryos (36-74 hpf, Fig. 3I, J, M and N). Expression of *shh*, an early marker of the differentiating notochord (Krauss et al., 1993), was not affected in the morphants (Fig. 3E-H). Careful examination of the notochord under high magnifications revealed that most of the control notochord cells were cylindrically shaped viewing laterally (Fig. 3K), whereas morphant notochord cells were of various shapes and sizes in both slightly affected embryos (Fig. 3L) and more severely affected embryos (Fig. 3O). Gaps were clearly visible among notochord cells in more severely affected embryos (Fig. 3O), whereas notochord cells in the control embryos were tightly associated (Fig. 3K). Injections of the 5-mis control MO resulted in embryos that were indistinguishable in morphology from uninjected control embryos (Table 1). Our results suggest that cadherin-7 is not required for the early differentiation of the notochordal cells, since the rod-like notochord is well formed by 12 hpf, hours before *cdh7*/Cdh7 expression is detected in the structure. The period of zebrafish cadherin-7 expression in the notochord (17 to 24 hpf) coincides with a critical stage of notochord development when chordamesoderm cells start to differentiate into mature notochord cells (Hawkins et al., 2008), therefore cadherin-7 is likely involved in this transition.

The notochord defects in the zebrafish *cdh7* morphant were different from those of *Xenopus* embryos with disrupted axial protocadherin function, in which separation of the notochord and other mesodermal cells (e.g. the pronephros) was severely affected. Notochord defects

in cadherin-2 mutants (Radice et al., 1997; Pujic and Malicki, 2001; Lele et al., 2003) have not been reported, although zebrafish *cadherin-2* mutants have crooked body axis (e.g. the body bends either to the left or right at 50 hpf in *glass onion* mutant, Pujic and Malicki, 2001), while mice cadherin-2 mutants display undulating neural tubes (Radice et al., 1997).

Expression of neural markers was not altered in the cdh7 morphant CNS

We previously showed that *cdh7* was expressed early (12 hpf), and exhibited a segmental pattern in the developing zebrafish CNS (Liu et al., 2007). To determine whether cadherin-7 regulates CNS differentiation, expression of several neural markers was examined in control and morphant CNS. *d1x2a*, a homeobox gene, is expressed as two diagonal bands, parallel to the optic recess, in the early embryonic forebrain (Akimenko et al., 1994). *dlx2a* expression pattern was similar between the control embryos (Fig. 4A) and *cdh7* morphants (Fig. 4E) at 24 and 36 hpf. *shh* and *pax7* are important dorsoventral patterning genes (Ericson et al., 1996; Matsunaga et al., 2001), and are known to regulate *cdh7* expression in chicken embryos (Luo et al., 2006). *shh* is expressed in the ventral region of the developing CNS (Krauss et al., 1993). Similar *shh* expression pattern was observed in the control embryos (Fig. 4B) and *cdh7* morphants (Fig. 4F). In embryonic zebrafish, *pax7* is expressed in almost the entire optic tectum and cerebellum, while its expression in the hindbrain and spinal cord is restricted to the border of the alar (dorsal) and basal (ventral) regions (Seo et al., 1998). Disruption of cadherin-7 function did not change this expression pattern in the morphants (24 hpf: Fig. 4C, D, G-K, M-O; 30 hpf, data not shown). To further examine development of the morphant hindbrain, expression of *krox20* (in rhombomeres 3 and 5, Oxtoby and Jowett, 1993) was compared between the control embryos (Fig. 4L) and *cdh7* morphants (Fig. 4P). Similar to expression of the other markers, there was no obvious difference in the *krox20* expression pattern between these two groups.

To examine cadherin-7 function in CNS development of older embryos (34-50 hpf), we performed zn5 (labeling a subset of differentiating cells in the CNS, Kanki et al., 1994; Sassa et al., 2007) and 3A10 (labeling a subset of hindbrain spinal cord projecting neurons such as Mauthner neurons, Brand et al., 1996) immunostaining. Zn5 expression domain size and shape, and zn5 positive cell projection patterns throughout the CNS were similar between control and *cdh7* morphants (Fig. 5). The Mauthner cells in the morphant hindbrain (Fig. 5T) also showed similar location, size, shape and projection pattern as those in control embryos (Fig. 5N). Despite the undulating spinal cord, likely due to the undulating notochord, zn5 positive cells (secondary motoneurons in the spinal cord, Fashena and Westerfield, 1999) in the morphant spinal cord were confined to the ventral 1/3 of the structure (Fig. 5G and S) as in the control embryos (Fig. 5C and M), and the periodicity of the spinal nerves was not disrupted (Fig. 5G and H). These results suggest that differentiation of the zebrafish CNS was not apparently affected in *cdh7* morphants. This may partially due to activities of other cadherins (e.g. cadherin-2) that compensate for the loss of cadherin-7 function in the morphant, as cadherin function redundancy is evident in mammals (Junghans et al., 2005).

Acknowledgements

We thank Dr. Eric Glasgow (Georgetown University) for providing cDNAs for zebrafish *dlx2a* and *shh*, Dr. Lisa Maves (Fred Hutchinson Cancer Research Center) and Dr. Hee Chan Seo (University of Bergen) for cDNAs coding for zebrafish *krox20* and *pax7*, respectively. This study was supported by NIH EY13879 (Q. Liu) and NIH DC006436 (J.A. Marrs).

References

Akimenko MA, Ekker M, Wegner J, Lin W, Westerfield M. Combinatorial expression of three zebrafish genes related to distal-less: part of a homeobox gene code for the head. J Neurosci. 1994; 14:3475–3486. [PubMed: 7911517]

Beckers A, Alten L, Viebahn C, Andre P, Gossler A. The mouse homeobox gene Noto regulates node morphogenesis, notochordal ciliogenesis, and left right patterning. Proc Natl Acad Sci USA. 2007; 104:15765–15770. [PubMed: 17884984]

Brand M, Heisenberg C-P, Jiang Y-J, et al. Mutations in zebrafish genes affecting the formation of the boundary between midbrain and hindbrain. Development. 1996; 123:179–190. [PubMed: 9007239]

Bumcrot DA, McMahon AP. Sonic signals somites. Current Biol. 1995; 5:612–614.

Down M, Power M, Smith SI, Ralston K, Spanevello M, Burns GF, Boyd AW. Cloning and expression of the large zebrafish protocadherin gene, Fat. Gene Expr Patt. 2005; 5:483–490.

Ekker SC. Morphants: a new systematic vertebrate functional genomics approach. Yeast. 2000; 17:302–306. [PubMed: 11119307]

Ericson J, Morton S, Kawakami A, Roelink H, Jessell TM. Two critical periods of sonic hedgehog signaling required for the specification of motor neuron identity. Cell. 1996; 87:661–673. [PubMed: 8929535]

Fashena D, Westerfield M. Secondary motoneuron axons localize DM-GRASP on their fasciculated segments. J Comp Neurol. 1999; 406:415–424. [PubMed: 10102505]

Glickman NS, Kimmel CB, Jones MA, Adams RJ. Shaping the zebrafish notochord. Development. 2003; 130:873–887. [PubMed: 12538515]

Gumbiner BM. Regulation of cadherin-mediated adhesion in morphogenesis. Nat Rev Mol Cell Biol. 2005; 6:622–634. [PubMed: 16025097]

Halpern ME, Ho RK, Walker C, Kimmel CB. Induction of muscle pioneers and floor plate is distinguished by the zebrafish *no tail* mutation. Cell. 1993; 75:99–111. [PubMed: 8402905]

Hawkins TA, Cavodeassi F, Erdélyi F, Szabó G, Lele Z. The small molecule Mek1/2 inhibitor U0126 disrupts the chordamesoderm to notochord transition in zebrafish. BMC Dev Biol. 2008; 8:42. [PubMed: 18419805]

Junghans D, Haas IG, Kemler R. Mammalian cadherins and protocadherins: about cell death, synapses and processing. Curr Opion Cell Biol. 2005; 17:446–452.

Kanki JP, Chang S, Kuwada JY. The molecular cloning and characterization of potential chick DM-GRASP homologs in zebrafish and mouse. J Neurobiol. 1994; 25:831–845. [PubMed: 8089660]

Krauss S, Concordet JP, Ingham PW. A functionally conserved homolog of the Drosophila segment polarity gene hh is expressed in tissues with polarizing activity in zebrafish embryos. Cell. 1993; 75:1431–1444. [PubMed: 8269519]

Kuroda H, Inui M, Sugimoto K, Hayata T, Asashima M. Axial protocadherin is a mediator of prenotochord cell sorting in *Xenopus*. Dev Biol. 2002; 244:267–277. [PubMed: 11944936]

Lele Z, Folchert A, Concha M, Rauch G-J, Geisler R, Rosa F, Wilson SW, Hammerschmidt M, Bally-Cuif L. parachute/n-cadherin is required formorphogenesis and maintained integrity of the zebrafish neural tube. Development. 2002; 129:3281–3294. [PubMed: 12091300]

Levi G, Ginsberg D, Girault JM, Sabanay I, Thiery JP, Geiger B. EP-cadherin in muscles and epithelia of Xenopus laevis embryos. Development. 1991; 113:1335–1344. [PubMed: 1811947]

Liu Q, Sanborn KL, Cobb N, Raymond PA, Marrs JA. R-cadherin expression in the developing and adult zebrafish visual system. J Comp Neurol. 1999; 410:303–319. [PubMed: 10414535]

Liu Q, Babb SG, Novince ZM, Doedens AL, Marrs JA, Raymond PA. Differential Expression of cadherin-2 and cadherin-4 in the developing and adult zebrafish visual system. Vis Neurosci. 2001a; 18:923–933. [PubMed: 12020083]

Liu Q, Marrs JA, Chuang JC, Raymond PA. Cadherin-4 expression in the zebrafish central nervous system and regulation by ventral midline signaling. Dev Brain Res. 2001b; 131:17–29. [PubMed: 11718832]

Liu Q, Duff RJ, Liu B, Wilson AL, Babb-Clendenon SG, Francl J, Marrs JA. Expression of *cadherin10*, a type II classic cadherin gene, in the nervous system of the embryonic zebrafish. Gene Expr Patt. 2006; 6:703–710.

Liu B, Duff RJ, Londraville RL, Marrs JA, Liu Q. Cloning and expression analysis of *cadherin7* in the central nervous system of the embryonic zebrafish. Gene Expr Patt. 2007; 7:15–22.

Luo J, Ju MJ, Redies C. Regionalized cadherin-7 expression by radial glia is regulated by Shh and Pax7 during chicken spinal cord development. Neurosci. 2006; 142:1133–1143.

Matsunaga E, Araki I, Nakamura H. Role of Pax3/7 in the tectum regionalization. Development. 2001; 128:4069–4077. [PubMed: 11641229]

Melby AE, Warga RM, Kimmel CB. Specification of cell fates at the dorsal margin of the zebrafish gastrula. Development. 1996; 122:2225–2237. [PubMed: 8681803]

Melby AE, Kimelman D, Kimmel CB. Spatial regulation of floating head expression in the developing notochord. Dev Dyn. 1997; 209:156–165. [PubMed: 9186051]

Nakagawa S, Takeichi M. N-cadherin is crucial for heart formation in the chick embryo. Dev Growth Differ. 1997; 39:451–455. [PubMed: 9352199]

Nakagawa S, Takeichi M. Neural crest emigration from the neural tube depends on regulated cadherin expression. Development. 1998; 125:2963–2971. [PubMed: 9655818]

Nasevicius A, Ekker SC. Effective targeted gene ‘knockdown’ in zebrafish. Nat Genet. 2000; 26:216–220. [PubMed: 11017081]

Nollet F, Kools P, van Roy F. Phylogenetic analysis of the cadherin superfamily allows identification of six major subfamilies besides several solitary members. J Mol Biol. 2000; 299:551–572. [PubMed: 10835267]

Odenthal J, Haffter P, Vogelsang E. Mutations affecting the formation of the notochord in the zebrafish, Danio rerio. Development. 1996; 123:103–115. others. [PubMed: 9007233]

Oxtoby E, Jowett T. Cloning of the zebrafish *krox-20* gene (*krx-20*) and its expression during hindbrain development. Nucleic Acids Res. 1993; 21:1087–1095. [PubMed: 8464695]

Parsons MJ, Pollard SM, Saude L, Feldman B, Coutinho P, Hirst EMA, Stemple DL. Zebrafish mutants identify an essential role for laminins in notochord formation. Development. 2002; 129:3137–3146. [PubMed: 12070089]

Pujic Z, Malicki J. Mutation of the zebrafish glass onion locus causes early cell nonautonomous loss of neuroepithelial integrity followed by severe neuronal patterning defects in the retina. Dev Biol. 2001; 234:454–469. [PubMed: 11397013]

Radice GL, Rayburn H, Matsunami H, Knudsen KA, Takeichi M, Hynes RO. Developmental defects in mouse embryos lacking N-cadherin. Dev Biol. 1997; 181:64–78. [PubMed: 9015265]

Redies C, Takeichi M. Cadherins in the developing central nervous system: an adhesive code for segmental and functional subdivisions. Dev Biol. 1996; 180:413–423. [PubMed: 8954714]

Sassa T, Aizawa H, Okamoto H. Visualization of two distinct classes of neurons by gad2 and zic1 promoter/enhancer elements in the dorsal hindbrain of developing zebrafish reveals neuronal connectivity related to the auditory and lateral line systems. Dev Dyn. 2007; 236:706–718. [PubMed: 17279576]

Seo HC, Saetre BO, Havik B, Ellingsen S, Fjose A. The zebrafish Pax3 and Pax7 homologues are highly conserved, encode multiple isoforms and show dynamic segment-like expression in the developing brain. Mech Dev. 1998; 70:49–63. [PubMed: 9510024]

Simonneau L, Broders F, Thiery JP. N-cadherin transcripts in *Xenopus laevis* from early tailbud to tadpole. Dev Dyn. 1992; 194:247–260. [PubMed: 1286211]

Smith JC. Dorso-ventral patterning in the neural tube. Current Biol. 1993; 3:582–585.

Stein S, Kessel M. A homeobox gene involved in node, notochord and neural plate formation of chick embryos. Mech Dev. 1995; 49:37–48. [PubMed: 7748788]

Takeichi M. Cadherin cell adhesion receptors as a morphogenetic regulator. Science. 1991; 251:451–5.

Talbot W, Trevarrow W, Halpern ME, Melby AE, Farr H, Postlethwaite JH, Jowett T, Kimmel CB, Kimelman D. Requirement for the homeobox gene *floating head* in zebrafish notochord development. Nature. 1995; 378:150–157. [PubMed: 7477317]

Turner DL, Weinstraub H. Expression of achaete-scute homolog 3 in *Xenopus* embryos converts ectodermal cells to a neural fate. Genes Dev. 1994; 8:1434–1447. [PubMed: 7926743]

Von Dassow G, Schmidt JE, Kimelman D. Induction of the *Xenopus* organizer: Expression and regulation of *Xnot*, a novel FGF and activin-regulated homeo box gene. Genes Dev. 1993; 7:355–366. [PubMed: 8095482]

Westerfield, M. The zebrafish Book. University of Oregon Press; Eugene, OR: 2000.

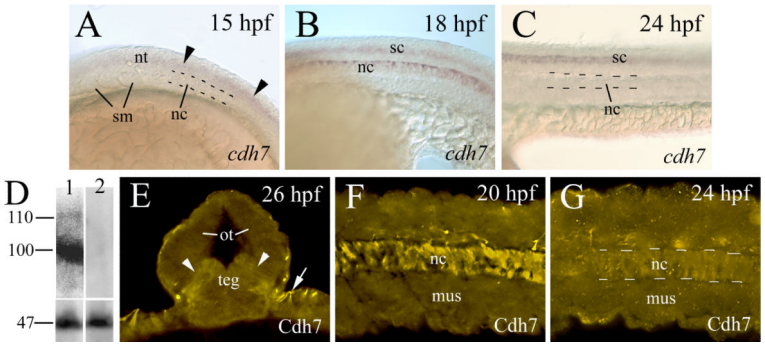

Figure 1.

Cadherin-7 message (*cdh7*, panels A-C) and protein (Cdh7, panels F and G) expression in the developing zebrafish notochord. Panels A-C show lateral views of the mid-trunk region of whole mount embryos (anterior to the left and dorsal up), while panels F and G are horizontal sections of the mid-trunk region (anterior to the left). Panel E is a cross section from the midbrain region (dorsal up). Arrowheads in panel A indicate *cdh7* expression in the neural tube (nt). The notochord (nc) in panels A, C and G is indicated by dashed lines. Cdh7 expression in panel E is indicated by two arrowheads. The arrow in panel E points to staining of an unidentified protein. Panel D shows immunoblot using 30-36 hpf whole mount embryo lysate demonstrating specificity of the affinity purified zebrafish cadherin-7 antibody. Staining of both the 100 kDa and 110 kDa molecular mass bands were eliminated when the antibody (lane 1) had been preincubated with excess cadherin-7 synthetic peptide used to generate the antibody (lane 2). The molecular mass band at 47 kDa, detected using the anti-acetylated tubulin antibody, shows the same amount of protein (40 μg total protein/ lane) was loaded. Additional abbreviations: mus, body trunk muscles; ot, optic tectum; sc, spinal cord; sm, somites; teg, tegmentum;.

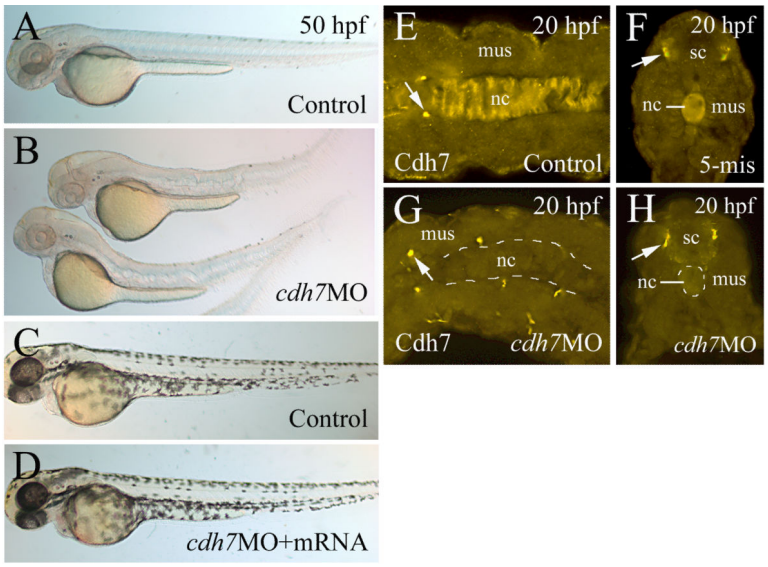

Figure 2.

Panels A and B show gross morphological defects in *cdh7* morphants. Panels A-D are lateral views of live embryos (anterior to the left and dorsal up) with embryos in panels A and B developed in PTU treated fish tank water, while embryos in panels C and D developed in normal fish tank water. *cdh7* morphants show undulating body trunks (panel B). Embryos injected with cdh7MO2 and *cdh7* mRNA (75-150 pg/embryo) show normal gross morphology (panel D). Panels E-H show that Cdh7 expression in the *cdh7* morphant notochord was greatly reduced (panels G and H) compared to that of an uninjected control embryo (panel E) or an embryo injected with the control (5-mis) MO (panel F). Panels E and G are horizontal sections (anterior to the left), while panels F and H are cross sections (dorsal up) of mid-trunk region. The notochord in the morphants is outlined by dashed lines. Arrows point to staining of the unidentified protein, which is similar between the control embryos and morphants. Abbreviations are the same as in Figure 1.

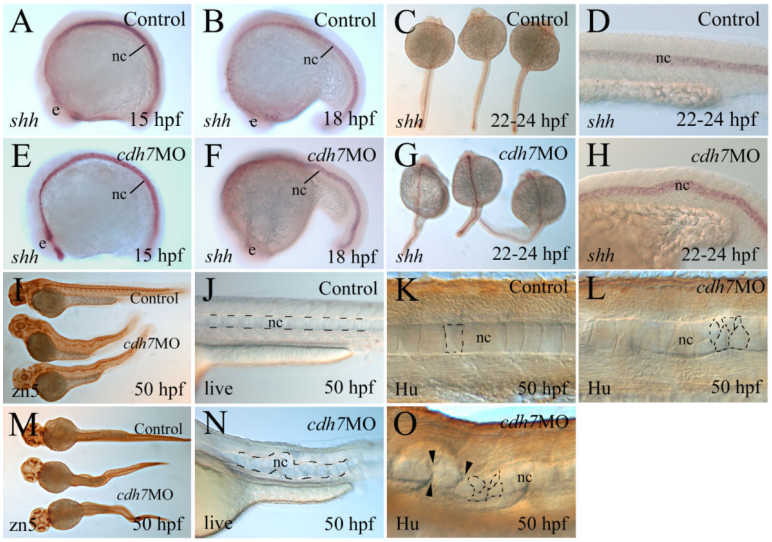

Figure 3.

Notochord defects in *cdh7* morphants. All panels are from whole mount embryos, with panels C (uninjected control embryos) and G (morphants) showing dorsal views (anterior up), panel M showing ventral views (anterior to the left), while the remaining panels showing lateral views (anterior to the left and dorsal up). Panels A-H are from embryos processed for *shh* in situ hybridization. Panels D and H are higher magnifications of the mid-trunk region showing *shh* expression in the notochord. Panels I and M show embryos processed for zn5 immunostaining. Panels J and N are higher magnifications of the mid-trunk region from live embryos. The notochord is indicated by the dashed lines. Panels K, L and O are higher magnifications of the mid-trunk region from embryos processed for anti-HuC/HuD (Hu) immunostaining. Outlines of a few individual notochord cells in these panels are indicated by the dashed lines. Arrowheads in panel O point to gaps among the notochord cells. Additional abbreviation: e, eye primordium. Other abbreviations are the same as in Figure 1.

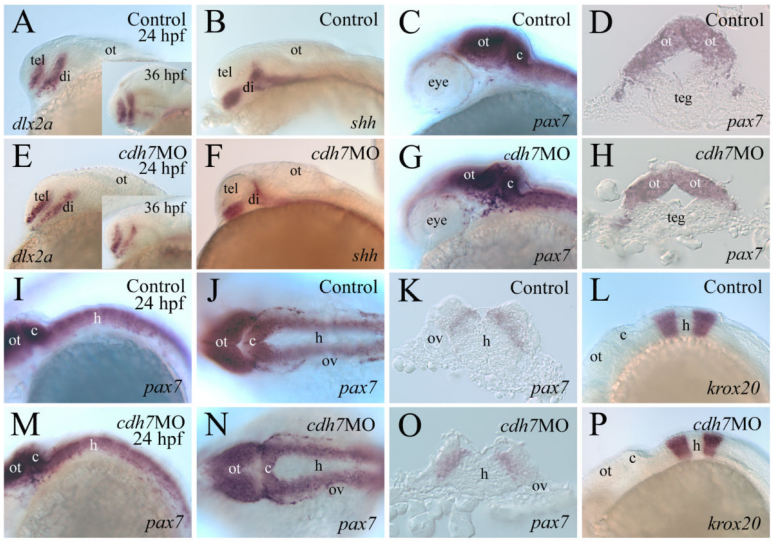

Figure. 4.

Expression of neural markers in the central nervous system is similar between control embryos and *cdh7* morphants. All panels, except panels D, H, K and O, are from whole mount embryos. Panels A-C and E-G show lateral views (anterior to the left and dorsal up) of the fore- and midbrain regions. Panels D and H are cross sections (dorsal up) at the midbrain level. Panels I, L, M and P are lateral views (anterior to the left and dorsal up) of the hindbrain region. Panels J and N are dorsal views (anterior to the left) of the hindbrain. Panels K and O are cross sections (dorsal up) at the otic vesicle level. Abbreviations: c, cerebellum; di, diencephalon; h, hindbrain; ot, optic tectum; ov, otic vesicle; teg, tegmentum; tel, telencephalon.

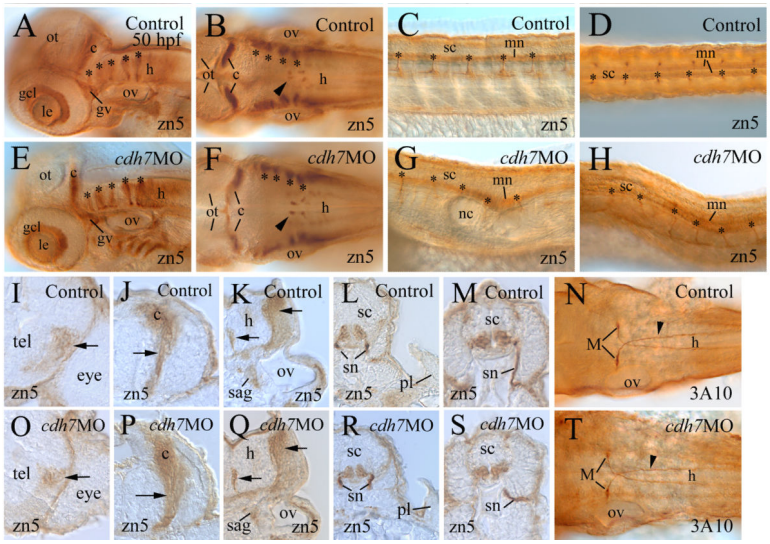

Figure 5.

Staining of zn5 (panels A-M, and O-S) and 3A10 (panels N and T) is similar between control embryos and *cdh7* morphants. Panels A-H and N and T are from whole mount embryos. Panels A and E are lateral views of the brain (anterior to the left and dorsal up), while panels B, F, N and T are dorsal views (anterior to the left) of the hindbrain region. Asterisks in these panels indicate zn5 positive vertical bands in the lateral hindbrain. The arrowhead in panels B and F point to several zn5 positive cells in the ventromedial hindbrain, while the arrowhead in panels N and T indicates Mauthner cell axons projecting to the spinal cord. Panels C and G are lateral views (anterior to the left and dorsal up) of the mid-trunk region. Panel D shows dorsal views (anterior to the left) of the mid-trunk region, while panel H shows a dorsolateral view (anterior to the left) of the mid-trunk region. Asterisks in these panels indicate locations of segmented spinal nerves. The remaining panels are from cross sections (dorsal up) at the telencephalon (panels I and O), cerebellum (panels J and P), otic vesicle (panels K and Q), pectorial limb bud (panels L and R) and mid-trunk (panels M and S) levels. Arrows in these panels point to similar zn5 positive cell clusters and their processes in control embryos and morphants. Abbreviations: gcl, retinal ganglion cell layer; gv, trigeminal ganglion; h, le, lens; M, Mauthner cells; mn, motoneurons; sag, statoacoustic ganglion; sn, spinal nerve. Other abbreviations are the same as in above figures.

Table 1

Effects of cdh7MOs injection on zebrafish development

	Number of embryos with undulating notochord (%)		Number of embryos examined at 50 hpf
Uninjected control	0		278
cdh7MO1 (3.4-6.8 ng)	53 (36.3%)		146
cdh7MO2 (3.4 ng)	61 (45.9%)		133
cdh7MO2 (6.8 ng)	129 (72.1%)		179
5-misMO (3.4-6.8 ng)		6 (3.1%)*	192
cdh7MO2 (6.8 ng) + *cdh7* mRNA (75-150 pg)	5 (6.2%) **	2 (2.5%)*	81

* These embryos had smaller and truncated bodies that were different from the *cdh7* morphants.

** The body defects in these embryos were milder than *cdh7* morphants.

Table 2

Effects of cdh7MO injections on zebrafish brain and spinal cord development revealed by ISH or ICC

	dlx2a	***krox20***	***pax7***	***shh***	**zn5**	**3A10**
22-24 hpf						
Control	n=20 (0%)	n=20 (0%)	n=25 (0%)	n=20 (5%)	ND	ND
cdh7 morphants	n=26 (7.7%)	n=20 (0%)	n=30 (0%)	n=25 (8%)	ND	ND
30-36 hpf						
Control	n=15 (13%)	ND	n=20 (5%)	n=28 (10.7%)	ND	n=16 (12.5%)
cdh7 morphants	n=15 (6.7%)	ND	n=18 (5.6%)	n=25 (16%)	ND	n=10 (10%)
43-50 hpf						
Control	ND	ND	ND	ND	n=25 (8%)	n=7 (14.3%)
cdh7 morphants	ND	ND	ND	ND	n=23 (13%)	n=6 (0%)

n, number of embryos examined; %, percentages of obviously reduced staining (staining area and/or intensity) compared to the majority of control embryos. All *cdh7* morphants at 30 hpf and older had undulating bodies. Abbreviations: ICC, immunocytochemistry; ISH, in situ hybridization; ND, analysis not done.